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CONTROL OF HEART RATE IN CECROPIA MOTHS; RESPONSE TO THERMAL STIMULATION

BY JAMES L. HANEGAN*
Department of Biology, Eastern Washington State College, Cheney, Washington, U.S.A.

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INTRODUCTION

It has been demonstrated that the large nocturnal moth, *Hyalophora cecropia*, maintains a stable elevated thoracic temperature during flight (Hanegan & Heath, 1970*a*). Preceding flight, a specialized activity of the flight musculature ('warm-up') elevates the thoracic temperature to favourable levels (Dorsett, 1962; Kammer, 1968). During flight these moths exhibit both behavioural and metabolic changes to maintain the thoracic temperature within narrow limits.

Heinrich (1970*a*, *b*) has shown that cardiovascular responses are altered to regulate the thoracic temperature of the sphinx moth, *Manduca sexta*. The abdominal heart rate responded directly to thoracic heating. The increased blood flow at high thoracic temperatures conducts heat from the warm thorax to the cooler abdomen and prevents overheating. The response is neurally mediated since cutting the ventral nerve cord abolishes the response.

In the present study, the responses of the heart of the moth to external heating and thermal clamping of the thoracic ganglia are reported. The response of the heart to thermal stimulation is discussed in terms of flight activity and the thermoregulatory mechanisms of these insects.

METHODS AND MATERIALS

Two different approaches are utilized in this study. First, measurement of heart rate during external heating of the thorax and abdomen; second, measurement of heart rate during thermode heating–cooling of the pterothoracic ganglia.

Externally heated animals. The legs of adult moths were glued to a small brass cup supported on a Plexiglass rod. The dorsal scales of the third abdominal segment were gently rubbed off so that the heart could be observed through the cuticle. Two small silver wires were pushed through the cuticle and rested on the surface of the heart. The electrical potentials of the heart were recorded differentially with a Tektronix 122 pre-amplifier and displayed on a Tektronix 502 oscilloscope. Permanent records of the potentials were made by recording the output of the oscilloscope on a polygraph.

The thorax and abdomen were heated separately by focusing a high-intensity microscope lamp on their surfaces. The temperatures of the thorax and abdomen during heating were monitored by use of copper-constantan thermocouples connected to a Honeywell recording potentiometer (Electronik-16).

* Mailing address: NASA Ames Research Center, Mail Stop 239–4, Moffett Field, California, U.S.A.

Thermode preparations. To prepare the moths for recording heart potentials the wings were removed and the scales on the dorsal scutum were gently rubbed off. The animal was then glued to a small Plexiglass plate ventral side up. The legs including the coxa and the ventral spina were removed to expose the thoracic ganglia. A small silver wire flattened at the tip was used to support the ganglia and also functioned as a thermode. A micromanipulator was used to position this wire beneath the pterothoracic ganglia which were then carefully lifted above the thoracic cavity without damaging the connecting nerves or the ganglia themselves.

The silver wire was inserted into the end of a plastic 'Y' tube which was connected to a water reservoir and a collecting flask via plastic tubing. Hot or cold water from the reservoir passed through the 'Y' tube either heating or cooling the silver wire. The water in the reservoir flask was maintained at a constant temperature ($\pm 0{\cdot}5$ °C) in a thermostatically controlled water bath. The temperature of water used for heating the ganglia varied from 40 to 48 °C, and 15 °C water was used for cooling. Using this thermode preparation the temperature of the pterothoracic ganglia could be maintained independently of the thoracic and abdominal temperatures.

Following the positioning of the thermode wire, a small incision was made in the third or fourth abdominal segment just dorsal to the tracheal openings. A small capillary tube (1·2 mm diameter) tapered and fire-polished to a small open tip (approximately 0·1 mm) was inserted through the incision and manipulated until the open tip was on the surface of the dorsal heart. Care was taken to prevent damaging either the dorsal heart or the ventral nerve cord. The capillary tube was connected by PE 50 tubing to a 1 c.c. tuberculin syringe filled with Cecropia saline (McCann, 1969). A small amount of saline was passed from the capillary to insure that the tip remained open. Suction was then applied using the syringe and a small portion of the dorsal heart was drawn up into the capillary tube. This suction electrode was connected to a Tektronix 122 pre-amplifier and the heart potentials were displayed on a Tektronix 502 oscilloscope. The animal was grounded through a silver wire placed in the abdominal cavity. Permanent records were made by recording the oscilloscope output on a polygraph.

RESULTS

External heating. In the whole-animal preparations external heating (2–4 °C/min) was used to control the thoracic or abdominal temperatures. Heart rate, monitored as a function of either the thoracic or the abdominal temperature, is shown in Fig. 1. This represents accumulated data for several heating and cooling cycles of one animal. The points represent heart rate measured over 30 sec intervals. In these experiments the abdominal temperature rose 2–3 °C with an initial time lag of 2–4 min after initiation of thorax heating. When the abdomen alone was heated, the thoracic temperature rose 3–5 °C with a time lag of 1–3 min after initiation of the heat pulse.

There does not appear to be a high correlation between heart rate and temperature. This is due, in part, to the erratic resting rate of the Cecropia heart. The heart exhibits bursts of impulses (2–6) followed by periods of inactivity. Additionally, the heart may show long periods of inactivity (10–30 sec) followed by a period of rapid pulsing.

In four of the six animals tested the heart rate appeared to be related to the state

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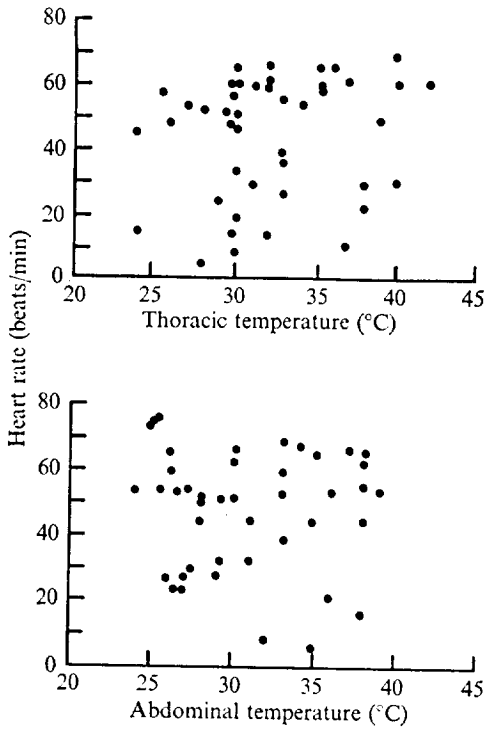

Fig. 1. Response of the abdominal heart rate to external heating of the thorax (upper graph) and external heating of the abdomen (lower graph).

of activity of the animal rather than to the temperature of the thorax or of the abdomen. When the animal was active (attempting flight) the heart rate tended to be high regardless of the thoracic temperature. Measurements of flight-muscle activity were not made on these animals so no direct correlation between activity and heart rate can be established.

However, in the thoracic temperature responses (Fig. 1) there appears to be a distinct break in the record which may reflect the activity state of the animal. The higher heart rates grouped at 40–70 beats/min may indicate an active animal while those rates below 40/min are representative of a torpid or inactive animal.

Heating–cooling of pterothoracic ganglia. Using the dissected moth preparation, the abdominal heart rate was monitored with a suction electrode while the pterothoracic ganglia were heated or cooled with a silver wire thermode. Heating was initiated when the animal was quiescent and the thoracic and abdominal temperatures were equal to ambient levels (24 °C).

Fig. 2 is a composite record of three moths in which the pterothoracic ganglia were heated. In record A the resting heart rate was slow and irregular (14 beats/min). Within 6 sec after the thermode was turned on the heart rate increased to 180 beats/min which was the highest rate observed in these experiments. The thermode temperature was 48 °C. In record B the resting heart rate was very regular at 24 beats/min. The rate increased to 70 beats/min within 3 sec after the start of heating. The thermode temperature was 40 °C. The heart rate remained stable at this level throughout the duration of heating. In record C the resting heart was pulsing in bursts of six followed

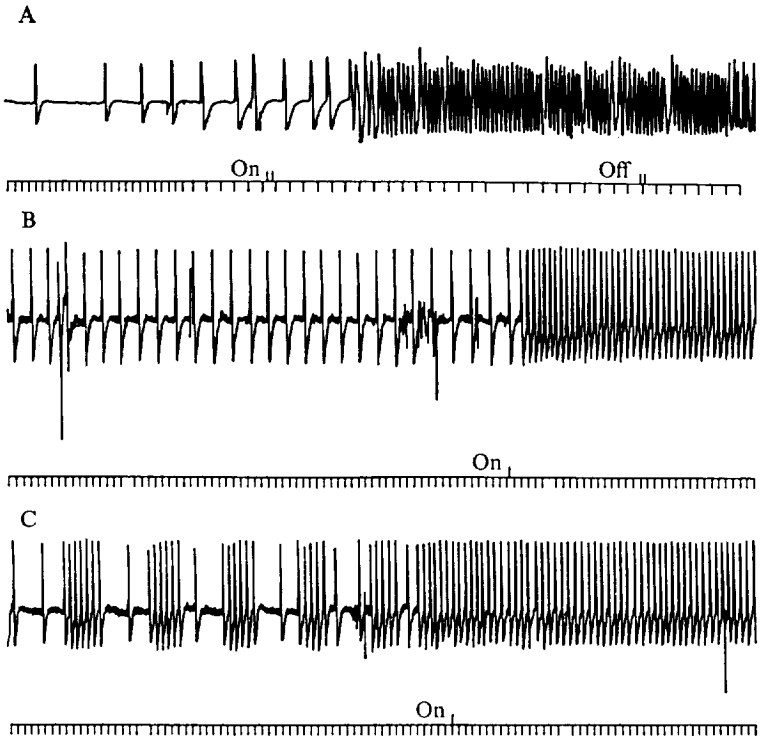

Fig. 2. Response of the abdominal heart to heating the pterothoracic ganglia with a thermode. The thermode temperature was 48 °C in record A and 40 °C in records B and C. Note the bursts of impulses in record C before heating was initiated. The time marks below the records indicate 1 sec intervals. The initiation of heating is indicated by the word 'on' next to the signal marks.

by an isolated beat. Thermode stimulation (40 °C) was initiated during a burst, and the heart started beating at a rate equal to that of the previous bursts, which was 60/min.

Fig. 3 is a record of three animals in which the pterothoracic ganglia were cooled. In record A the heart rate was high (66 beats/min) on the left side of the record due to previous heating of the ganglia. At the time mark cooling was initiated and approximately 25 sec later the heart rate slowed to 36 beats/min. With continued cooling the heart began to beat in bursts similar to those seen in the resting rate of Fig. 2C.

In record B the heart rate was spontaneously higher than normal resting rates, and heating was not used to stimulate the heart. When cooling was initiated, the heart rate immediately (3–5 sec) slowed from 40 to 25 beats/min. On the left side of record C the heart rate was elevated (140 beats/min) due to prior heating of the ganglia. Cooling was started at the point indicated and approximately 13 sec later the heart rate began to slow, and 30 sec later the heart was almost completely inhibited.

To determine whether heating–cooling of the pterothoracic ganglia altered the abdominal heart rate through humoral agents or through direct neural connexions, the ventral nerve cord was cut at the first abdominal segment. With the ventral cord cut, heating of the pterothoracic ganglia had no effect on abdominal heart rate (Fig. 4A). Heart rate fluctuates in animals with the ventral nerve cord severed, however, and during a period of spontaneously elevated heart rate cooling of the

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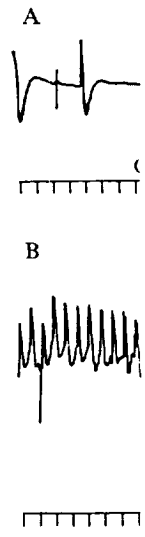

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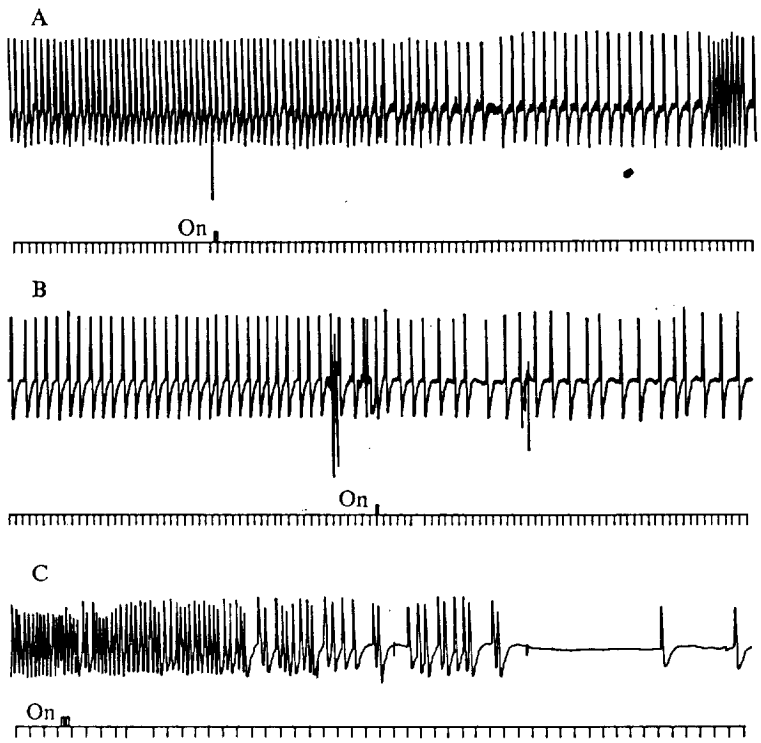

Fig. 3. Response of the abdominal heart to cooling the pterothoracic ganglia with a silver wire thermode. In records A and C the high rates on the left side of the records are due to previous thermode heating. In record B the resting rate on the left side of the record was spontaneously high. Note the almost complete inhibition of the heart in record C after cooling was started. The initiation of cooling is indicated by the signal mark and the word 'on'. Time marks indicate 1 sec intervals.

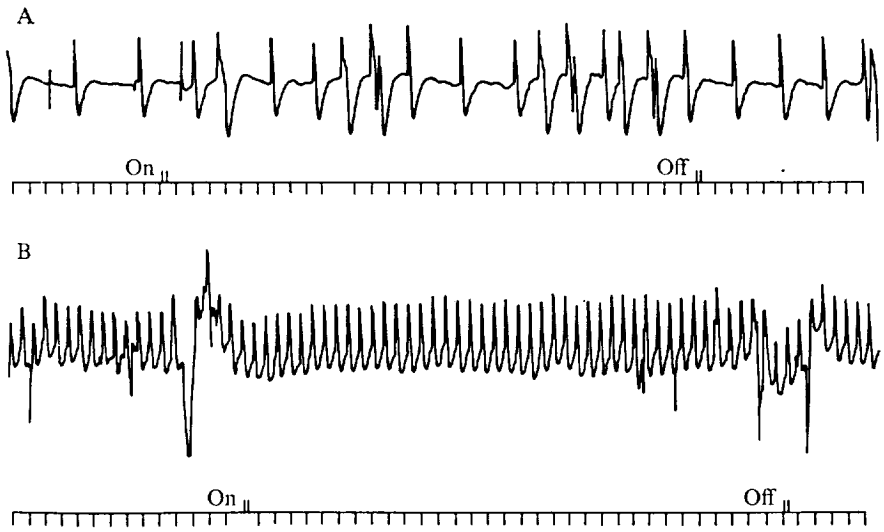

Fig. 4. Responses of the abdominal heart to heating (record A) and cooling (record B) the pterothoracic ganglia with the ventral nerve cord severed. Initiation of heating/cooling indicated by the 'on' next to the signal mark. Time marks indicate 1 sec intervals.

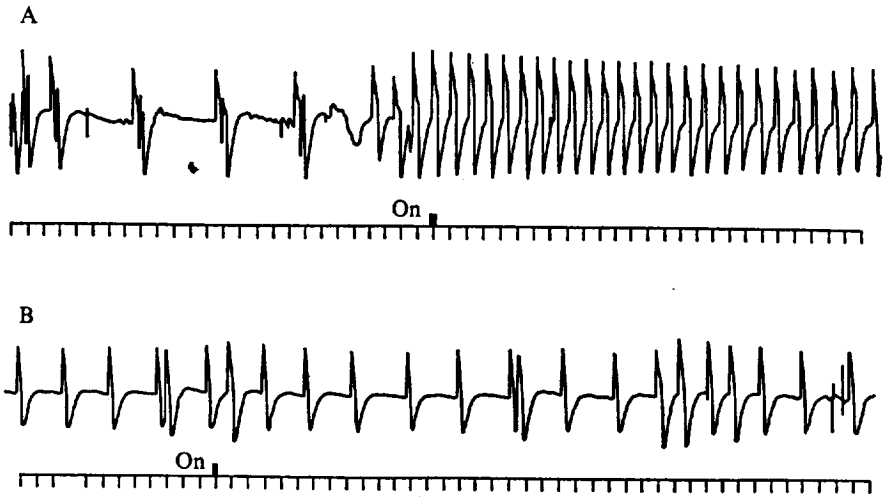

Fig. 5. Response of the abdominal heart to perfusion of saline at 38 °C (record A) and saline at room temperature (Record B). The ventral nerve cord was cut in these experiments. Start of saline perfusion at 'on'. Time marks are 1 sec intervals.

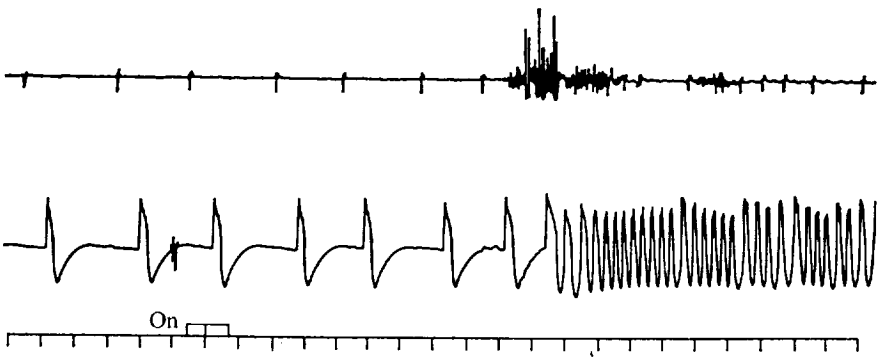

Fig. 6. Record of flight-muscle response (upper trace) and heart response (lower trace) to thermode heating of the pterothoracic ganglia. The small potentials seen in the upper trace are due to the electrodes in the flight muscle picking up the electrical activity of the dorsal blood vessel as it passes through the thorax. The start of thermode heating is indicated by the 'on' next to the signal mark. The time marks indicate 1 sec intervals.

ganglia was initiated (Fig. 4B). In this case cooling of the ganglia had no effect in altering the heart rate..

Animals in which the ventral nerve cord had been cut were subjected to abdominal perfusion of heated saline to test the direct response of the heart to temperature changes. When 0·5 ml of saline at 38 °C was injected into the abdomen, the heart rate rapidly increased with a short lag time (Fig. 5A). Injections of saline at room temperature (24 °C) had no effect on heart rate (Fig. 5B). The heart, isolated from connexions to the thoracic ganglia, exhibited the normal response of increasing rate with increased temperature.

Visual observations of animals during measurement of heart rate as functions of abdominal and thoracic temperatures indicated that heart rate may be positively coupled to the flight system. To investigate this phenomenon further, fish-hook electrodes were placed in the dorsal longitudinal muscle (DLM) which produce the

downstroke of the wings during flight (Hanegan, 1972; Hanegan & Heath, 1970*b*). These electrodes also recorded electrical activity of the dorsal heart as it passes through the thorax (upper trace of Fig. 6). When the pterothoracic ganglia were heated, a burst of activity in the DLM preceded the increased heart rate (lower trace). The muscle activity stopped within a few seconds but the heart rate continued at the higher rate for the duration of thermal stimulation.

DISCUSSION

External heating of the thorax of Cecropia moths does not cause a predictable rise in abdominal heart rate. Heart rate appears to be independent of the thoracic temperature. Visual observations and simultaneous records of heart rate and flight-muscle activity (Fig. 6) indicate that the heart rate is a function of the activity state of the animal.

Heating the abdomen of intact animals, in which the thoracic temperature reached a maximum of 3–5 °C above ambient temperature, had a variable effect on heart rate. In dissected preparations of Cecropia moths the isolated abdominal heart rate increases with increasing temperature (McCann, 1969). Similar conditions were achieved in this study by cutting the ventral nerve cord. In these animals perfusion of warm saline (38 °C) caused a marked increase in heart rate. Saline at a room temperature (24 °C) had no effect. It is significant that in these animals thermal stimulation of the thoracic ganglia had no effect on abdominal heart rate (Fig. 4).

In animals with the ventral nerve cord intact thermal stimulation of the pterothoracic ganglia directly controls heart rate (Fig. 2). Heating of the ganglia causes an increased heart rate. Thermode temperature is critical in these experiments. The higher the temperature of the thermode, the greater the magnitude of the heart response, with a maximum of 180 beats/min at a thermode temperature of 48 °C. Lower thermode temperatures (40 °C) cause the heart rate to increase up to 60–80 beats/min which is similar to the range observed when the thorax is heated externally.

Thermode heating of the thoracic ganglia, in addition to altering heart rate, alters the activity state of the animal. The change in activity from torpor to warm-up or flight is strictly dependent upon the temperature of the thoracic ganglia (Hanegan, 1972; Hanegan & Heath, 1970*b*). The initiation of the heart response follows the activation of the flight muscles (Fig. 6). Therefore, once flight or warm-up is activated, the abdominal heart responds by an elevated rate.

These findings support those of Heinrich (1971) using the sphinx moth, *M. sexta*. Heating the thorax of this moth with an external source did not cause an elevation of heart rate until the thoracic temperature reached 34–35 °C. At this critical temperature the heart rate began to increase with a continuing increase in thoracic temperature. The initiation of the heart response at 34–35 °C corresponds to the minimal thoracic temperature for flight in Cecropia moths and is within the range of temperature for thermode stimulation of the ganglia producing the flight pattern.

In animals with the ventral nerve cord cut the heart rate was significantly lower and remained constant with increasing thoracic temperatures (Heinrich, 1971). There is no neural input from the thoracic ganglia to the abdominal heart when the nerve cord is cut. Therefore, in terms of heart response, the animal is inactive even though

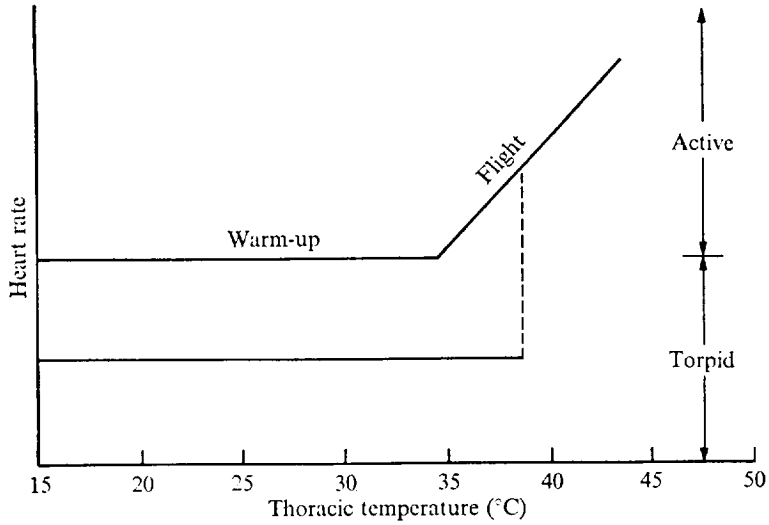

Fig. 7. A model of the heart responses to thermal stimulation in Cecropia moths. Activity is indicated on the right side of the graph. Two general forms of activity are considered, warm-up and flight, which are labelled. The heart rate is the Y axis on the left side of the graph. Absolute values are not listed because of variability among individuals; an upward deflexion indicates an increased rate. Further details of the model are discussed in the text.

it may be in warm-up or flight, and the local pacemaker is driving the heart at a basal rate.

A model to account for regulation of heart rate must take into consideration the following: (1) the isolated heart has its own pacemaker activity and is sensitive to changes in temperature, (2) heart rate during activity (flight or warm-up) is dependent upon neural control from the pterothoracic ganglia, and (3) the controller for heart rate in the pterothoracic ganglia is temperature-sensitive, the higher the temperature of the ganglia during activity the higher the heart rate.

A model for the heart responses of Cecropia moths is shown in Fig. 7. At an ambient temperature of 25 °C both thorax and abdomen would be at 25 °C when the animal is inactive. As the animal changes from an inactive to an active state, the heart rate increases to the elevated level indicated as warm up. The thoracic temperature at this point is 25 °C, which is too low to permit flight, so the animal activates the warm up mechanism in which the flight muscles contract synchronously producing heat. This activity results in elevation of the thoracic temperature. As the thoracic temperature increases, the heart rate remains relatively stable until it reaches the critical level for flight (approximately 35–37 °C). The increased temperature of the thorax acts on the thoracic ganglia to change the motor output from warm-up to flight. Continuous flight can result in a continued increase in thoracic temperature. Heart rate increases due to an increasing thoracic temperature and heat conduction from the warm thorax to the cooler abdomen becomes greater. The thoracic temperature is stabilized by the increased conductive heat loss as demonstrated by Heinrich (1971).

The initial rise in heart rate when the animal makes the transition from inactivity to warm up would be predicted as a consequence of the increased metabolic demands

placed on the system by the flight muscles. In adult Cecropia moths the flight muscles utilize long chain fatty acids but store very little neutral fat or free fatty acids (Domroese & Gilbert, 1964). The lipid substrate for flight muscle metabolism is stored in the abdominal fat bodies. It is released to the haemolymph in the diglyceride form and transported by one of the haemolymph proteins to the active tissue (Chino & Gilbert, 1965).

The initial heart response could thus meet the metabolic demand of increased activity. The rate change would not alter heat conduction pathways significantly to prevent warm up. Heat loss through conduction depends on the temperature difference between the warm thorax and the cool abdomen, and at the initiation of activity (warm-up) this difference is at a minimum. Only when the animal is fully warmed and the temperature sensitive ganglia cause further increases in heart rate does heat loss through conduction become important as a thermoregulatory mechanism.

The model does not account for stroke volume changes which are known to occur with increasing heart rate (Heinrich, 1971). Heath *et al.* (1971) assumed a constant heart rate of 100 beats/min and calculated stroke volume changes needed to maintain a constant thoracic temperature in *Manduca sexta*. The stroke volume must increase from 0·1 μl to 2·0 μl at a flight speed of 6 m/sec and from 0·2 μl to 4·0 μl at 8 m/sec. These volume changes are large and may not be realistic since heart rate changes with thoracic temperature. The pathway for conductive heat loss necessary for maintenance of a constant thoracic temperature is probably dependent upon both heart rate and stroke-volume changes.

SUMMARY

1. The site for regulation of heart rate is the pterothoracic ganglia.

2. Heart rate increases when the animal goes from an inactive to an active state. At low air temperatures the transition is from torpor to warm-up and above 35 °C the transition is from torpor directly to flight.

3. The heart of Cecropia moths isolated from neural connexions to the thoracic ganglia responds directly to temperature changes.

4. In actively flying animals the heart rate increases in response to thoracic heating.

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